

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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PHO
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In re the Application of:

Robert G. LAMB

Application No.: 09/670,346

Filed: September 27, 2000



Attorney Dkt. No.: 021941-00001

For: VITAMIN E PHOSPHATE/PHOSPHATIDYLCHOLINE LIPOSOMES TO PROTECT FROM OR AMELIORATE CELL DAMAGE

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

February 2, 2001

Sir:

Prior to calculation of the filing fee and prior to the examination of this application, please amend the above-identified application as follows:

In the Specification:

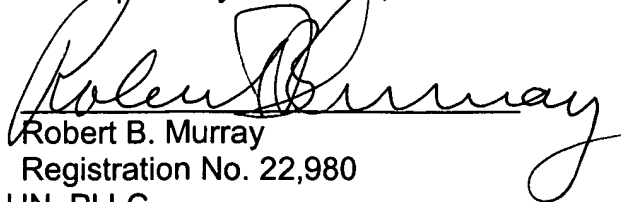
Please delete page 5 and substitute the attached new page 5.

REMARKS

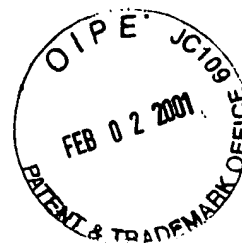
The above amendment has been made to correct the formatting of the chart in Example 2. No new matter has been included.

Please charge any fee deficiency or credit any overpayment to Deposit Account No. 01-2300.

Respectfully submitted,


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1.25 ml insulin (100 units/ml.)

10 ml pyruvic acid stock solution (11.22 g/100 ml)

Example 1:

Liposomes containing the calcium salt were prepared. Phosphatidylcholine (200 mg) was dissolved in 5 ml of DMSO. 200 mg of the vitamin E phosphate (calcium salt) was added. The mixture was sonicated 5 min. at 37°C degrees. Fifteen ml of 0.9% saline was added. The mixture was then sonicated for 15-30 minutes at 37°C.

Example 2:

Influence of vitamin E phosphate/phosphatidylcholine liposomes (see example 1) on Allyl alcohol-induced liver injury was evaluated in male albino mice.

Treatment	SGPT
	% of control \pm SEM
Control (9) vehicle only	100 \pm 7
Allyl alcohol (9)	330 \pm 15 **
Vehicle plus VEP/PC (9)	100 \pm 3 **
<u>Allyl alcohol plus VEP/PC (9)</u>	<u>109 \pm 4 **</u>

VEP/PC is vitamin E phosphate/phosphatidylcholine

** The mice were exposed to a single intraperitoneal dose of allyl alcohol (50 mg/kg) or vehicle for 4 hours.

Example 3:

Compositions using the sodium salt of the vitamin E phosphate were prepared in the following manner:

To 1 part (10 mg) of vitamin E phosphate sodium salt was added 4 parts (40 mg) of phosphatidylcholine. There was added sufficient sterile water to yield a total volume of 5 ml. The composition was then sonicated at 37°C for 10 to 15 minutes. The preparation was then sterilized by irradiation. The liposomes formed using the sodium salt proved to be more preferred than either the microcrystals or the liposomes prepared using the calcium salt of the vitamin E phosphate.

The use of vitamin E as disclosed in the prior art as an agent to protect cells from toxic injury has shown little or no promise for use as a therapeutic in vivo. It is now seen that the phosphate ester of the vitamin, when formulated in a manner that prevents hydrolysis by esterases in the gut and serum, can be used to protect cells from toxic injury in vivo. When treating the intact animal, any technology that delivers the phosphate ester of the vitamin E to the tissues subject to damage from oxidative stress such as, for example, exposure to toxins, including those occasioned by therapeutic agents, will be appropriate. The use of liposome technologies to protect the active agents provides a useful means of delivery.

A particularly useful function of vitamin E phosphate/phosphatidylcholine liposomal compositions of the present invention is to prevent or treat liver cell damage caused by the admini-